Effects and Action Mechanism of Low Level Laser Therapy (LLLT): Applications in Periodontology

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Abstract

Lasers nowadays have several applications in many research fields. In the life sciences and especially in Periodontology, Low Level Laser Therapy (LLLT) has proven to be particularly interesting. Low intensity radiation in specific wavelengths has been known to trigger cellular proliferation and differentiation, through molecular mechanisms that are still not fully elucidated. These effects might be triggered by mitochondria and through photobiological-photochemical phenomena caused by laser radiation, energy densities, time, and irradiation conditions as well as the appropriate individual settings in the various laser devices, which will predictably lead to optimal therapeutic effects, without providing a clarified view. What is generally accepted is that the energy density required is very small, at the level of 2-4 J/cm² and the transmit power is less than 0.5 Watts.

It appears that the bio-stimulant activity in tissues relates to short wavelengths, since the best results are displayed in red and near-infrared light spectrum. It is striking that, as per several descriptions, LLLT application has even achieved restoration of neural tissue injuries of the spine [1]. It is also highly interesting that studies have proved the beneficial effects of LLLT in the healing of peripheral nerve injuries and the regeneration of neuro-axons of injured nerves in animal models [2,3], as well as iatrogenic numbness improvement of the inferior alveolar nerve in humans [4].

Introduction

Nowadays, Lasers have a multitude of applications, ranging from mechanical to life sciences. In the field of Periodontology, Lasers are mostly known for their application in the removal of tissues or hemostasis through their ability to enhance the cellular processes throughout the applied energy in terms of space, time, and surface.

Cells by exerting its photochemical phenomena. Despite this, the mechanism of action and the effects of LLLT. We also aim to highlight how different wavelengths and energy applied can have a distinct impact on the effectiveness of LLLT. Finally, we provide insight on its current and potential future applications.

Keywords: Cells; Growth factors; Lasers; Mitochondria

Abbreviations: LLLT: Low Level Laser Therapy; NO: Nitric Oxide; COX: Cytochrome C Oxidase; ROS: Reactive Oxygen Species; ATP: Adenosine Triphosphate; AP-1: Activator Protein 1; GF: Growth Factor; PDGF: Platelet-Derived Growth Factor; TGF-β: Transforming Growth Factor Beta; IGF: Fibroblast Growth Factor; IGf: Insulin-like Growth Factor; BMP: Bone Morphogenetic Protein; PGE2: Prostaglandin E2; IL-1β: Interleukin-1b; IFN-γ: Interferon gamma; Nd-YAG: Neodymium-doped Yttrium aluminium garnet; KTP: Potassium Titanyl Phosphate; COX-2: Cyclooxygenase-2; MAPK: Mitogen Activated Protein Kinase

The photobiological-photochemical phenomena caused by laser radiation to the tissues, are similar to photosynthesis carried out by plants. To enable the visible light of low energy to affect any living biological system, the energy-carrying photons must be absorbed by electrons belonging to a photoreceptor or chromophore of the target biological system [5]. It has been theorized that the radiation red or near-infrared spectrum, causes stimulation of mitochondria [6]. The photoreceptor molecules or widely known as chromophores are molecules or portions of molecules which confer a particular color to the molecule or substance to which they belong. The chromophores are either conjugated electron transport systems or metal complexes. Characteristic examples of chromophores are observed in chlorophyll, hemoglobin, Cytochrome Oxidase C (COX), myoglobin, flavoproteins and porphyrins [7]. The photoreceptor molecules of mitochondria, on which laser radiation seems to act as a photo-stimulant, can trigger a series of photo-chemical reactions, which in turn, can cause changes in cellular metabolism, such as protein signaling. The key photoreceptor
molecule is COX, the last enzyme in the electron transport chain of mitochondria. Those photoreceptor molecules are NADPH oxidase, Nitric Oxide (NO), cytochrome aa3Cytochrome aa3 quinol oxidase, subunit III (respiratory chain component) while the key photoreceptor is the molecule of COX (a key molecule of the mitochondrial respiratory chain) (Figure 1) [8]. NO which is generated in the mitochondria of the cells when they are under stress or oxygen deficiency conditions, interferes with respiration because it antagonizes the oxygen in connection with COX [9]. It is described that LLLT application in cell cultures or in experimental models, results in an increase of NO concentration. This is because laser radiation causes release of NO by mitochondria and COX [10]. The result is the lifting of the respiratory interference that had caused the connection of NO with COX, because the flow of oxygen is restored, so that once respiration has re-started, ROS are produced [11].

The oxygen radicals (singlet oxygen molecules) (ROS), as free radicals play an important role in the formation of Adenosine Triphosphate (ATP), which in turn is the energy storage of the cell [12]. LLLT results in respiratory re-start through the release of NO by iron ions and copper COX, oxygen recapture, production of ROS and ATP production increase. It should be noted that the release of NO under the influence of low-power laser radiation, is affected by other cell energy storages such as hemoglobin and myoglobin molecules which capture NO [13].

Formation of proteins, the activation of enzymes and the cell cycle promotion [17]. Finally, LLLT causes change in the biochemical cell activity due to the transient heating of the chromophore molecules. This happens with the small dose of energy, minimally supplied, if there is no increase in tissue temperature [17] (a slight increase of 1°C is attributed to the activation of cellular metabolism).

As it has been clarified, the primary reactions taking place in the mitochondria are followed by a series of chain reactions that occur in the cell resulting in the modification of cellular homeostasis. So, change is observed in the pH, in the concentration of intracellular Ca2+ [11], in the ATP and the cAMP as well as in the redox potential. These changes at the cellular level have been hypothesized to explain the clinical observation of a series of positive effects of LLLT, such as increased cell proliferation, accelerating the healing process, promoting tissue regeneration, preventing cell death, analgesic action, relieve of neurogenic pain (laser acupuncture), anti-inflammatory activity etc. The synthesis of DNA and RNA and the increase of cell proliferation is induced by the increase of the intracellular Ca2+ concentration provoked by LLLT activity on the Ca2+ channels [18].

Despite what has been said above regarding the mechanisms by which LLLT exerts its bio-stimulatory action, the exact mechanism that causes analgesia has not been identified, it has been argued that the laser light selectively stimulates the free nerve endings, which are located in the superficial layers of the skin and mucous membranes. Their effect is based on mechano-thermal phenomena which in some way affect the nociceptive afferent nerve fibers [19,20], changing their activity and suppressing neurostimulators resulting from the pain receptors of peripheral nerves [21]. The analgesic effect of LLLT however, has been supported by systematic reviews of both medical [22,23] and dental fields [24].

A special mention is worth to the effectiveness of LLLT in the treatment of dentinal hypersensitivity. A success in pain relief reaching the percentage of 90% has been reported. This interesting effect is
probably exerted through inhibition of the depolarization of C pulp fibers [25,26].

Low doses of energy induce all these favorable effects. When high doses of energy are administered at the same wavelength, the results are least favorable or even catastrophic for the cell. This is called biphasic response to the radiation dose [17]. The larger power supply, however (e.g., by increasing the time of exposure, or the energy density), will lead to warming, depletion of mitochondrial respiratory chain and ultimately cell death. Providing even more energy by increasing the intensity, reducing the pulse time, and increasing the frequency in the device, would lead to photoagulation and photo-sublimation of the irradiated tissue [18,27,28]. In applying radiation of low energy density, it has been clearly shown that very good therapeutic effects in tissues that were damaged are achieved (cells not operating normally). In contrast, when applied on healthy cells, or tissues in which inflammation was artificially induced, LLLT did not bring such good results [29,30].

Low Level Laser Therapy (LLLT) and Healing of Tissues

During the healing process, there is a series of well-orchestrated and controlled events with which the body is trying to achieve regeneration of damaged by a traumatic or infectious cause tissue, both morphologically and functionally. Healing begins with inflammation that is created after the injury or the exercise of another damaging cause. Initially, various types of cells are chemotactically attracted to the injured area and phagocytize tissue remnants, bacteria, and impurities. Subsequently, new extracellular matrix is created, which functions as a scaffold for cell built up, creating vascular neoplasms, and finally bridging and reuniting the trauma edges. The complete healing is achieved with the maturation of the newly formed tissue. If the newly formed tissue is identical structurally and functionally with the background, regeneration of tissue has been successfully achieved. If the new tissue is not structurally or functionally identical to its predecessor, then we are talking about reparation [31]. Healing performed after a trauma or other cause, is not the same in all cases. It differs per type of cause, extent of damage, type of affected tissue etc.

After the effect of a damaging cause (trauma, surgery, infection, burn, etc.) and the creation of damage which extends beneath the epithelium to the underlying connective tissue, the result is injury to the blood vessel which subsequently causes fibrin formation and platelet thrombus. Then the activation of various cells begins within the trauma and into the adjacent tissue [31]. The sequence of the well-orchestrated events that follow, such as chemotaxis, proliferation, cell differentiation, and the composition of the intercellular matrix, is controlled and regulated by biological mediators, called Growth Factors (GFs). These are molecules of polypeptide nature, whose influence on the tissue healing are similar for all types of tissues, including the periodontal one [32].

The main GFs involved in the healing process are: The Platelet-Derived Growth Factor (PDGF), the Transforming Growth Factor Beta (TGF-β), the acidic and basic Fibroblast Growth Factor (aFGF) and (bFGF respectively), the Insulin-Like Growth Factors (IGF-I and -II) and the Bone Morphogenetic Proteins (BMPs).

Cells such as platelets, fibroblasts, vascular endothelial cells, and immune cells, play a key role in the healing process. Through a complex sequence of events, chemotaxis, proliferation, and differentiation of cells finally result in tissue healing. A few years after the laboratory preparation of laser beam, the scientists tried to take advantage of the ability of the radiation effect on living matter and investigate the relationship between LLLT and trauma healing [33]. First, the Hungarian physician, Endre Mester from Semmelweis University, used the term bio-stimulation and studied the effect of LLLT laser-rubidium on animals. After the use of radiation on mice, which had been shaved on the back and then irradiated with low intensity radiation, it was found firstly that the mice did not develop tumor lesions and, secondly, that the flanks of the mice in the irradiation group grew faster compared to the group not irradiated [34].

Since then, numerous studies on a cellular level, laboratory animals, and in humans have confirmed the beneficial effect of laser treatment on wound healing. It has been argued that the best time to apply LLLT is at the stage of cell proliferation [35,36]. It has been shown that LLLT can cause increased bFGF production, which exerts mitogenic activity and induces angiogenesis and tissue repair [37,38]. Furthermore, after applying LLLT an increase of cell proliferation of gingival fibroblasts and bFGF and IGF-1 release [39], have been demonstrated as well as increased protein levels and the corresponding mRNA of biological IL-la mediators and IL-8 [40]. In other studies, an increase of the secretion of VEGF, bFGF, HGF and SCF GFs was found, which also promotes the proliferation of fibroblasts [41]. Increased production of Prostaglandin E2 (PGE2) through the introduction of Cyclooxygenase-2 (COX-2) mRNA has also been described [42,43]. The effect of PGE2 in the periodontal tissue cells is dose-dependent and at low concentrations it appears to contribute to the reformation. In international literature, there is a plethora of research data that support the positive effect of LLLT on cell proliferation [39,43,44]. Besides the increase in cell proliferation, it has been argued that LLLT may promote cell differentiation as well. For example, transformation of fibroblasts into myofibroblasts has been found [45].

However, far fewer investigations have focused on identifying the intensity of the provided radiation as well as the energy density which would safely and predictably result in the bio-stimulation of cells rather than in their destruction [46,47]. Perhaps the heterogeneity between different conditions and irradiation methods used in different surveys is the reason for which other researches have produced opposite views - that is, that LLLT application offers no benefit to the healing process. For example, some researchers have failed to achieve a statistically significant difference in healing time, in animals or humans [48-50].

This was the result of a systematic review of the available studies on the effect of LLLT in the treatment of osteoarthritis. It was concluded that well-organized studies with the corresponding laser and irradiation conditions, from which firm conclusions could be drawn on the role and therapeutic doses of LLLT, were absent [51]. The different results between 'similar' studies can be attributed to irradiation with inadequate or, on the contrary, excess energy, to the use of incorrect frequency on irradiation of inadequate or incorrect affected area etc. [52]. In another systematic review of the in vitro studies and the studies on animals concerning LLLT, the authors concluded that the data from studies on animals are insufficient to generalized conclusions as to the reliability of the therapeutic approach in humans [53]. Even though, there is a lot of research data and a number of systematic reviews [54], which successfully demonstrate the beneficial effect of LLLT on trauma healing [55], at reducing pain [56], in strengthening neo-angiogenesis [56], in healing vesicular stomatitis lesions [57], in treating mucositis of irradiated patients at the oropharyngeal region [58], etc., further, well-designed surveys are
required to clarify the whole thing, especially the doses and conditions of irradiation [59].

In periodontal literature, several studies are reported on the effect of LLLT on fibroblasts of the connective tissue [60], to the periodontal ligament cells [61] and osteoblasts of the alveolar and jaw bones [62].

Enough evidence supports the beneficial effects of laser radiation on the proliferation and activation of fibroblasts. Also, a dose-dependent reduction in the production of PGE2, by decreasing the expression of COX-2 has been argued [63]. Moreover, it has been demonstrated that LLLT affects, in a dose dependent manner, on gingival fibroblasts, preventing the production of Interleukin-1b (IL-1b) [64]. The very fact that LLLT has provided a decrease in the expression of cytokines IL-1b and Interferon gamma (IFN-γ), as well as an increase in the expression of GFs (PDGF, TGF-b, bFGF), leads to the interesting conclusion that lasers exert anti-inflammatory activity and promote healing [64]. The positive effect on healing has been demonstrated through an in vitro study of LLLT effect (with KTP or Nd-YAG Laser) on a primary culture of skin fibroblasts, in which increased collagen production, increased secretion of antioxidant enzymes and increased vinculin expression appeared (adhesion molecules expressed by endothelial cells [65,66]. The LLLT with diode laser also has a positive effect in the healing of the gingiva, showing to lead to increased mRNA production of GFs VEGF and TGF-β and collagen type 1 [67].

The knowledge about the success of the reconstructive procedure with the application of LLLT, gave thought to its application to promote regeneration of damaged periodontal tissues. It has been supported that the ability of LLLT to suppresses the plasminogen activator production of COX-2, phospholipase A2 (enzymes responsible to produce PGE2), PGE2, IL-1b prevents the degradation of the extracellular matrix since it reduces collagen destruction that occurs when the cells are found under stress conditions [68]. Furthermore, the beneficial effect of LLLT on the proliferation of periodontal ligament cells has been strongly supported [69-71], whereas other investigators have questioned it [46]. Interesting is a study which evaluated the possible additional effect of LLLT in the implementation of protein matrix of the enamel (Emdogain®), in endostal injuries which were caused by periodontitis in humans. Although there was no statistically significant difference in the adhesion increase and the reduction of the depth of the follicles, significantly less recession was observed in patients treated with LLLT [72].

However, LLLT has also bio-stimulatory activity in cells of the bone tissue. It has been demonstrated that LLLT with Nd:YAG laser causes an increase in cell proliferation in cultured cell lines of osteoblasts [73]. In relatively recent experimental study on rats, Altan, et al. [74] demonstrated that LLLT causes acceleration of bone remodeling during orthodontic tooth movement, promoting the proliferation and function of osteoblasts and osteoclasts. The increase in cell proliferation was confirmed by studying the LLLT effect with Nd: laser YAG both in primary osteoblast cultures and in osteoblast cell line [62]. For the explanation of the phenomenon, activation of the Mitogen Activated Protein Kinase (MAPK) [62], increase of the BMP-2 expression of osteocalcin and TGF-b1 have been investigated and demonstrated [75]. Indeed, it has been shown that favorable results are obtained with the smallest possible doses [73].

Moreover, molecular techniques have confirmed the effect of LLLT and the diversification of pre-osteoblasts [76]. In studies regarding therapeutic intervention to treat intraosseous periodontal defects in humans, it was estimated that the effect of LLLT combined with alloplastic graft, with clinical and radiographic criteria in the first 3 postoperative months, was positive. However, after six months there was no difference with the control group. So, it was concluded that the LLLT worked in accelerating the healing process [77,78]. Indeed, it was found in vitro, that the LLLT increases alkaline phosphatase activity and mRNA expression of substances such as osteocalcin, bone sialoprotein and osteopontin, which are all indicators of osteoblastic differentiation [78].

In the field of implantology, animal studies suggested that LLLT with diode laser application on the bone of the recipient position has resulted in faster osseointegration and higher percentage of bone to implant contact. Moreover, it appeared that the torques required to export osseointegrated implants and the percentages of calcium and phosphorus (ratio by weight) in peri-implant bone were statistically higher in the group of animals that received LLLT. It was concluded that increased levels of calcium and phosphorus demonstrate faster bone maturation, hence faster [35,36]. By applying LLLT with diode laser in in vitro studies, it was shown that there was an increase in cell proliferation, increased adhesion of primary fibroblast cultures and osteoblasts on titanium surfaces, as well as an increase in cell proliferation and differentiation of tumor osteoblasts series (osteoblast like cells), and TGF-b and osteocalcin production by cells of tumor lines [76].

The favorable effect of LLLT in osseointegration has been indirectly supported by a study on experimental animals (rabbits) that received implants with reduced initial stability [79]. The same experimental model LLLT, again with a diode laser (780 nm), resulted in statistically significantly greater bone-implant contact, when ceramic implants were used, as well as increased bone micro-hardness [80].

Furthermore, in cultures of osteoblasts on titanium surface, which received low-power laser radiation, a decrease of the cell population was firstly observed, which soon reversed, while there was an increase of the alkaline phosphatase expression of osteocalcin and BMP-7 [81].

Almost all studies on the effect of laser radiation in osseointegration, relate to the bio-stimulatory action of laser diodes. The review of the literature on the effects of LLLT on cells of the bone tissue, with wavelengths in the infrared spectrum, leads to the conclusion that LLLT promotes cell proliferation of osteoblasts, increases collagen deposition, and creates new bone. It is also emphasized that the application of LLLT was proposed to be applied at the very early stages of healing, because then the cell proliferation potential is still high [82].

References

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